For Combat Wounded: Extremity Trauma Therapies From the USAISR

David I. Devore, PhD; Thomas J. Walters, PhD; Robert J. Christy, PhD; Christopher R. Rathbone, PhD; Joseph R. Hsu, MD; David G. Baer, PhD; Joseph C. Wenke, PhD

Our focus here is on the future role of the U.S. Army Institute of Surgical Research (USAISR) in reducing the morbidity associated with extremity combat wounds. These wounds are the most frequent and debilitating suffered in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF). There have been over 40,000 total American combat casualties in OEF and OIF, and of those severely injured on the battlefield, 82% had at least 1 musculoskeletal extremity wound.[1,2]

USAISR is a subordinate command of the U.S. Army Medical Research and Materiel Command (USAMRMC), and since 1947 it has been located at Fort Sam Houston, San Antonio, TX. The Army Burn Unit has been an integral part of USAISR since 1949. Research on trauma and orthopedics has been a part of USAISR's mission since 1970. The USAISR buildings are contiguous with Brooke Army Medical Center (BAMC), which enables the research organization to have a unique in-depth understanding of the combat injuries and the medical challenges faced by the clinicians who treat them.

Our mission is to optimize combat casualty care (CCC). In fulfilling this mission, USAISR provides requirements-driven CCC medical solutions and products for injured warriors from self-aid through definitive care. Research is currently organized in 9 Task Areas, i.e., Extremity Trauma and Regenerative Medicine, Damage Control Resuscitation, Pain Control, Advanced Capabilities for Emergency Medical Monitoring, Critical Care Engineering, Clinical Trials, Eye Trauma, Craniomaxillofacial, and Blood (Coagulopathy), to focus on the most critical aspects of combat wound care from the battlefield to upper echelon hospitals. Our core research program is funded and supported by the USAMRMC through 2 complementary Research Area Directorates, CCC and Clinical and Rehabilitative Medicine. We also receive funding from competitive peer-reviewed grant programs and biotechnology companies. The Extremity Trauma and Regenerative Medicine task area has the ultimate goal of returning the injured warrior to full function through an innovation process that starts with understanding the injuries and resultant clinical challenges; runs through laboratory research, product development, and translation to clinical trials; and ends with partnerships with industry to supply the finished products to clinicians and battlefield medics.

U.S. Army Institute of Surgical Research, Fort Sam Houston, San Antonio, TX 78234.

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DATA-DRIVEN OBJECTIVES

Our research objectives are determined by data-driven analyses of combat casualties using information from the Joint Theater Trauma Registry (JTTR). The majority of battlefield wounds are to the extremities. Because of improved battlefield trauma care and body armor, the overall mortality of those wounded in combat has declined from 23% in World War II to below 12% in OEF and OIF. With this reduced mortality has come increased severity and difficulty of treatment of the wounds. Most of the wounded experience polytraumatic injuries averaging 2.3 extremity wounds per injured warrior. This is a direct result of the high energy explosive devices used in the asymmetric combat of the current conflicts. The extremity wounds are evenly distributed between upper and lower extremities, with 53% being penetrating injuries to soft tissues and 26% fractures; 82% of those fractures are open wounds. Almost 18,000 warriors wounded in action have not been able to return to duty,[2] and extremity injury is the main reason for their limited functional recovery. Our analysis of JTTR data for patients evaluated by the U.S. Army Physical Evaluation Board indicates that orthopedic injuries are responsible for almost 70% of unfitting conditions that prevent return to duty, with lower extremity amputations and loss of nerve function having the highest impact based on frequency and degree of disability.[3] Hospital and disability costs for the treatment of extremity injuries exceed 60% of all costs for wounds suffered in OIF and OEF.[4] Infection and nonunion are major interrelated complications that cause frequent re-hospitalizations. To better identify the causes of poor clinical outcomes, we are now evaluating a database of over 200 open fractures with significant soft tissue injury (type III). Parameters that we are weighing include concomitant soft tissue loss, nerve defects, infection, and the type of fracture fixation devices used.

Although the JTTR effectively captures battlefield casualty data according to the injury mechanism, body region injured, and calculated injury severity using International Classification of Diseases, 9th Revision, Clinical Modification codes, these coded data fields lack the granularity required to answer some of the most pressing clinical questions, especially in the realm of orthopedic trauma. Currently, we have to use the patients' charts to gather the necessary information. The Military Orthopaedic Trauma Registry has, therefore, been

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE **01 JUN 2011**	2. REPORT TYPE **N/A**	3. DATES COVERED **-**

4. TITLE AND SUBTITLE
For combat wounded: extremity trauma therapies from the USAISR

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Devore D. I., Walters T. J., Christy R. J., Rathbone C. R., Hsu J. R., Baer D. G., Wenke J. C.,

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
United States Army Institute of Surgical Research, JBSA Fort Sam Houston, TX

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR’S ACRONYM(S)

11. SPONSOR/MONITOR’S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**UU**	**5**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

created by the JTTR, the Department of Orthopaedics and Rehabilitation at BAMC, civilian orthopedic experts, and USAISR to allow us to more rapidly collect and utilize the more detailed information that is needed to understand the severity of the extremity wounds, how they are treated, and their outcomes. The Military Orthopaedic Trauma Registry has access to the data records at BAMC and other major treatment facilities through an approved Institutional Review Board protocol.

USAISR is also an active partner with other Army, Navy, and Air Force organizations in the Joint Trauma Analysis and Prevention of Injury in Combat Project.[5] Joint Trauma Analysis and Prevention of Injury in Combat collates data provided by USAISR on combat injuries with operational data from other sources to form a systems approach by engineers and scientists working to develop improved solutions for the prevention or mitigation of blast-related injuries.

RESEARCH CONSORTIA FACILITATION

We are at an exciting point in time where advances in cell biology, biochemistry, biomaterials, and bioengineering make it feasible to address the enormously complex medical problems of severe polytrauma. But no one organization has the facilities or capabilities to solve these problems alone. USAISR is, therefore, facilitating major research consortia of the nation's leading scientists, engineers, and clinicians dedicated to repair and regeneration of combat wounds. Extremity trauma research objectives for the consortia are set based on the analysis of combat injury data from the JTTR and other sources.[6] One of these consortia is the Orthopaedic Extremity Trauma Research Program, a congressionally directed, peer reviewed research program that since 2006 has funded 26 translational research and small clinical studies.[7] Also, beginning in 2008, Orthopaedic Extremity Trauma Research Program has been supporting a large, multicentered consortium conducting prospective randomized clinical trials that offer the greatest likelihood of changing clinical practice and improving outcomes. USAISR and USAMRMC are managing this $18.4 million consortium, the largest such effort in orthopedic trauma research, with direction and input from Army, Navy, and Air Force orthopedic services. The School of Public Health at Johns Hopkins coordinates data for the consortia's 12 civilian and 4 Department of Defense medical treatment centers. Thirty other clinical centers are designated as satellite centers and will participate fully pending future funding. The consortia plans to conduct at least 3 prospective randomized clinical studies that address the range of clinical challenges from acute care through reconstruction of bone defects, fracture fixation, and treatment of infection. Information about the consortium is available at http://www.usaisr.amedd.army.mil/otrp.html and http://www.metrc.org.

USAISR is also the principal military research organization within the Armed Forces Institute of Regenerative Medicine (AFIRM), established in 2008 through the leadership of USAMRMC. AFIRM is a multidisciplinary network of leading scientists and physicians at 2 academic–industry consortia, the Rutgers University–Cleveland Clinic Consortium and the Wake Forest Institute of Regenerative Medicine–University of Pittsburgh McGowan Institute for Regenerative Medicine Consortium. AFIRM research groups have pioneered in the development and commercialization of stem cells, biomaterials, bioactive agents, and surgical protocols for regenerative medicine. The goal of AFIRM is to rapidly advance tissue engineering technologies to clinical trials for wounded warriors in 5 specific therapeutic areas identified with guidance from USAISR to be of highest priority: burn repair, scarless healing, limb and digit salvage, craniofacial reconstruction, and compartment syndrome. Several clinical trials have already been initiated by AFIRM, including 2 at USAISR/BAMC on autologous fat transfer for repairing burn scars and on xenograft extracellular matrix for skeletal muscle regeneration. Information on AFIRM is available at http://www.afirm.mil/.

BASIC AND APPLIED RESEARCH

At USAISR, our Extremity Trauma and Regenerative Medicine task area is building core competencies in physiology, stem cell biology, microbiology, and biomaterials to address the specific challenges of repairing and regenerating infected, burned, and avulsed tissues. We conduct translational preclinical research studies with in vitro methods and in vivo animal model protocols that we develop to mimic specific traumatic injuries. For example, we are adapting high-throughput in vitro microfluidic systems to determine if nanoparticle delivery of novel antimicrobial agents can effectively treat orthopedic biofilms. Our in vivo goat wound infection model allows us to establish clinical practice guidelines for irrigation of contaminated wounds,[8] to assess the effects of negative pressure wound therapy on bacterial contamination,[9] and to evaluate osteoinductive bone graft biomaterials that control delivery of antimicrobials.[10] From this work, 2 local antibiotic delivery devices have been licensed by biotechnology companies. We also have animal models for compartment syndrome[11] and large segmental muscle loss.[12] By routinely working in collaboration with leading academic researchers, we are able to advance such novel concepts as bioactive wound dressings for control of infection and pain[13] and dual-purpose biodegradable polyurethane–bone microparticle composites that promote bone regeneration while preventing infection.[14]

Infection is the leading cause of death in combat-related burn injuries,[15] and hence one of our primary research objectives is the development of novel antimicrobial systems. Infections cause exudation at the wound site that prevents healing and wound closure by reducing oxygen tension, degrading extracellular matrix proteins, and delaying epithelialization. We are developing novel drug delivery systems with diverse biomaterials to eliminate such infections. This currently includes a chitosan-based delivery system for silver sulfadiazine, a drug often used to treat burn infections, and polymer–liposome complexes for delivery of novel cationic antimicrobial peptides.

We are focusing our regenerative medicine research on applications of adult stem cells and biomaterial scaffolds for the repair of skin, muscle, and bone injuries. There is a rapidly expanding list of clinically supported benefits that adult stem cells can provide. For example, for patients with extensive burn areas, there is often not enough uninjured skin tissue to provide for extensive autologous skin grafts. We are developing engineered stem cell–based skin replacements using relatively plentiful adipose-derived mesenchymal stem cells (ASC). ASCs are easily isolated from the stromal vasculature of subcutaneous adipose tissue by liposuction, and the concentration of pluripotent stem cells is 100–1000 times greater than those in bone marrow. A number of hydrogel-based dressings are clinically available for burns and wound healing, and we are working with one of these, a fibrin gel modified with poly(ethylene glycol) (PEG), to provide a suitable scaffold for the proliferation of ASC. This PEGlated fibrin hydrogel can be used to control ASC differentiation toward vascular cell types in the absence of growth factor supplementation. This will allow us to develop both vasculature and dermal connective tissue from a single population of ASC. It is our goal to prepare layered composites of the ASC in the hydrogel as wound dressings or for vascularized dermal equivalents that can overcome the current limitations of nutrient diffusion in other tissue-engineered constructs.

The effect of the timing of stem cell delivery to a wound site is a key factor that we are evaluating. We are transplanting adult muscle precursor cells at various time points following skeletal muscle ischemia–reperfusion injury in an animal model. Our preliminary observations are that there is significantly improved muscle function when the injured muscles receive the muscle precursor cells at 2 days after injury, as demonstrated by greater muscle force compared to vehicle control. We are also finding that freshly isolated bone marrow-derived stem cells can home to injured muscle when injected systemically 2 days after the injury. These results are particularly interesting given the availability of point-of-care devices for bone marrow–derived stem cell isolation and delivery that might influence early treatment approaches for combat wounds in theater.

CLINICAL TRIALS

We work in partnership with the Department of Orthopaedics and Rehabilitation at BAMC to translate our extremity wounds research to the clinic. Together we have initiated several clinical trials in the areas of CCC and several more are planned in the near future. We are a study site for one of the largest and most militarily relevant clinical studies in the world, the Fluid Lavage of Open Wounds study, a multinational trial of irrigating solutions and pressures on open fractures.[16] Wound debridement and irrigation is the most common surgical procedure performed on injured warriors; therefore, the potential for this study to impact the care of our injured is great. BAMC will also be one of the study sites for the above mentioned Major Extremity Trauma Research Consortium. Fracture fixation, bone grafting, and infection studies will be the first studies initiated by this consortium. This will further develop needed infrastructure, allow military personnel to gain expertise, and will solidify a research culture within these orthopedic departments.

The prevention and control of infections in clinical procedures is central to our research efforts. Because infectious pathogens are often not detected rapidly enough with current microbiological culture methods, we are studying a new technology, the Ibis T5000, that can identify pathogens on hospital surfaces and in orthopedic wounds in just a few hours. The Ibis T5000 is a universal pathogen biosensor that couples nucleic acid amplification to high-performance mass spectrometry and base component analysis for quantitative identification of bacteria that have never been isolated or sequenced. The genes for antibiotic resistance, such as the *Mec A* that confers resistance to methicillin, are also detected by the Ibis technique so that methicillin-resistant *Staphylococcus aureus* can be differentiated from its methicillin-sensitive counterpart.

One of our recent clinical case studies exemplifies our biomaterials research direction. This case involves restoring lost muscle in the right thigh of a 19-year-old Marine who had substantial muscle weakness for 3 years post-injury. This is the first known repair of a large volumetric muscle loss using a surgical technique that applies an innovative multilayered scaffold composed of extracellular matrix derived from porcine intestinal submucossa.[17] The patient has had no complications and with physical therapy has made marked improvement in isokinetic performance consistent with the increased new muscle tissue at the implant site observed by computer tomography. This tissue engineering approach provides a new treatment option for extensive muscle loss injuries.

We are also actively engaged in an ongoing clinical project to recreate the energy and function lost with a dynamic ankle–foot orthosis. The Intrepid Dynamic Exoskeletal Orthosis (IDEO) is a carbon fiber, energy-storing brace created at the Center for the Intrepid in collaboration with BAMC and our research team. We are using it on the combat injured with muscle and nerve deficits below the knee. In addition to some encouraging clinical results,[18] we are enrolling patients in 2 comparative effectiveness research trials. The first trial evaluates functional measures with the IDEO vs. commercially available ankle–foot orthoses. The second trial is a biomechanical gait analysis aimed at optimizing strut stiffness for desired performance using the Military Performance Laboratory at the Center for the Intrepid.

PARTNERSHIPS FOR COMMERCIALIZATION

To accelerate product development, USAISR is actively developing Cooperative Research and Development Agreements (CRADA) in the areas of soft tissue and bone injury, infection, and tissue regeneration. We are providing technical oversight to more than 20 large research contracts with universities and companies. The CRADA provide intellectual property and confidentiality assurances for both parties. Our CRADA projects are responsible for the development of several novel medical devices, including a noninvasive, laser-based technology for in vivo assessment of skeletal muscle injury with continuous wave near infrared spectroscopy, commercially available enzyme-linked immunosorbent assay kits for skeletal muscle–specific troponin for common laboratory animals using purified biomarkers for assessment of skeletal muscle injury in serum and extracellular fluid, and scaffold-based approaches for the treatment of volumetric muscle loss. Recently initiated CRADA are focused on novel treatments for biofilm infections associated with orthopedic fracture fixation devices and on a new generation of bioactive nerve regeneration conduits for repair of critical size gaps. We are thus able to leverage established academic technologies like a rodent model for biofilms on fracture fixation pins and the product development expertise of biotechnology companies who have the manufacturing and commercialization capabilities needed to bring products with Food and Drug Administration approvals to clinicians. Because USAISR is a military research organization without manufacturing capacity, our industrial collaborations are essential for fulfilling our vital mission.

FOR COMBAT WOUNDED

Everything that the USAISR does is for combat wounded. We face unprecedented challenges in repairing and regenerating fully functional tissue for injured warriors who have suffered polytrauma injuries to their extremities. Our objective is to improve outcomes by using a 2-pronged approach: reduce initial complications (primarily infections and nonunions) and restore function to injured limbs by using regenerative medicine and tissue engineering solutions. Our projects have led to several major advances in clinical practice and many of our new projects are currently in the clinical trial stage. We are now conducting research on stem cells, bioactive agents, antimicrobials, and tissue regeneration scaffolds in collaboration with leading academic and industrial research groups. Our responsibilities extend beyond the laboratory both to coordinating major research consortia on orthopedic trauma and tissue regeneration and to leading clinical trials and product translation. Our new state-of-the-art research facility, the Battlefield Health and Trauma Research Institute, has just been opened, and we are poised to lead the way in CCC research.

ACKNOWLEDGMENT

Funding for the project was provided by the USAMRMC, Fort Detrick, MD.

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